Copper, β-amyloid, and Alzheimer's disease: Tapping a sensitive connection

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Redox chemistry arising from transition metals copper and iron are the principal chemical origin of the radicals and reactive oxygen species that are implicated in the pathogenesis of a number of degenerative diseases, such as Alzheimer's disease (AD) (1) and atherosclerosis (2). In health the brain strictly regulates the movement of these metals across the blood–brain barrier (BBB), which is relatively impermeable to fluctuations in blood levels. This barrier is relevant in AD because the disease is characterized by the accumulation in the brain of β-amyloid (Aβ) (3), a copper/zinc-metalloprotein that aggregates and becomes redox-active in the presence of excessive amounts of these metals (4, 5). We are only beginning to unravel the age-dependent failure of metal homeostatic mechanisms of the brain that contribute to abnormal Aβ biochemistry in AD (6).

In a recent issue of PNAS, Sparks and Schreurs (7) reported that the ingestion of low concentrations (≈2 μM) of copper in drinking water markedly impairs trace conditioning and increases neuronal and brain parenchymal Aβ immunoreactivity in cholesterol-supplemented rabbits. This finding is provocative because it implies that tap water has sufficient copper contamination to impact the pathophysiology of AD. Although intriguing, there are several caveats before concluding from these data that AD itself is fostered by environmental exposure to copper.

The authors report markedly increased Aβ-immunoreactive cortical neurons in the copper-treated rabbits. Cholesterol treatment alone induces an increase, which is significantly augmented by cotreatment with copper. Indeed, tap water treatment alone appears to induce more neuronal Aβ immunoreactivity than distilled water, an effect the authors link to copper contamination. The result suggests that Aβ metabolism is extraordinarily sensitive to small changes in copper concentrations that might be transduced across the BBB. This interpretation is supported by previous observations that both Aβ (5, 8) and the amino terminus of amyloid protein precursor (APP) (9) possess high-affinity, selective Cu^{2+} binding sites. A function for the Cu^{2+} binding site on Aβ is implied by the structural similarity of the coordinating residues with those of the femtomolar-affinity Cu^{2+} binding site on superoxide dismutase 1 (10, 11). The function of Aβ that might be subserved by this highly ordered, positively cooperative metal binding site is unclear, but recent reports have suggested that the peptide can sequester Cu^{2+} and prevent the metal ion from generating damaging reactive oxygen species (12, 13). This potential function is compatible with other recent findings that overexpression of mutant APP (Tg2576) or the carboxyl-terminal 100 aa of APP (CT100) decreases tissue copper levels in transgenic mice (6). Therefore, Aβ may be part of a metal clearance system, which becomes corrupted in AD (1). In this view, the increase in intraneuronal Aβ levels observed by Sparks and Schreurs (7) would be a reflection of an up-regulated homeostatic system attempting to lower elevated neuronal copper levels. In this model, Aβ production would be a normal neuronal homeostatic response to a copper challenge, whose corruption leads to incipient Alzheimer pathology.

We are only beginning to unravel the failure of metal homeostatic mechanisms in Alzheimer's disease.

Elevated cholesterol and elevated copper levels are both factors that have been proposed to lead to the conversion of Aβ from a functional peptide to a self-aggregating neurotoxin. The levels of copper in the brain rise markedly with aging (6), and we have proposed that the consequent abnormal decoration of Aβ with Cu^{2+} leads to two principal abnormal reactions: redox activity leading to H_2O_2 production (14, 15) and aggregation (5, 8). Aβ (Aβ1–42>Aβ1–40>rat Aβ) reduces Cu^{2+} to Cu^{+} in a catalytic reaction cycle that uses O_2 and biological reducing agents as substrates and generates neurotoxic H_2O_2 as the product (15). A peptide radical may form in this process, explaining how very low concentrations of Cu^{2+} induce oxidation and cross-linking of the peptide (8, 16). Although it is unlikely that the full 2-μM increase in Cu^{2+} that differentiates tap from distilled water will cross the BBB, increases in the nanomolar range could still impact significantly on the metabolism of Aβ, based on the affinity constants for Cu^{2+} binding (8). Therefore, an impact on cerebral Aβ metabolism by dietary Cu^{2+} concentrations of the caliber described by Sparks and Schreuers is plausible and may increase the risk of Aβ deposition.

Recent studies have observed both epidemiological and biochemical associations between elevated cholesterol and AD (17). The mechanism by which elevated cholesterol might act to foster abnormal Aβ reactivity is not certain, but possibilities proposed include the increased generation of Aβ (18, 19). In the rabbit model used by Sparks and Schreuers, cholesterol engenders increased neuronal Aβ immunoreactivity and "senile plaque-like structures." However, the Aβ immunoreactive collections that appear in the cortical parenchyma in the rabbits are not mature AD amyloid plaques. They lack both surrounding gliosis and neuritic involvement. Furthermore, there is no evidence of neurofibrillary tangle formation or synaptic loss. But AD research lacks a fully satisfactory animal model, and, despite its incompleteness, the Sparks and Schreuers copper/cholesterol model is worthy of further exploration. An important study in appraising its validity as a model for AD would be to determine whether the cortical changes in Aβ, the senile plaque-like structures, and the attenuated conditioning responses persist after the copper/cholesterol challenge is withdrawn. Lack of persistence of these changes would imply that the cholesterol/copper combination engenders an acute stress (comparable to delirium), in contrast to igniting a chronic

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degenerative AD-like condition (comparable to dementia).

An important feature of the copper/cholesterol challenge in this model is that the increase in neuronal Aβ immunoreactivity is accompanied by a marked deterioration in trace conditioning responses. Although this is not a cognitive change that meets all criteria for dementia, it does indicate that the rabbit could be cognitively obtunded. Therefore, the dietary combination of elevated cholesterol and slightly elevated copper appears to distress the brain. The mechanism for this is unclear, but the changes in blood superoxide dismutase, gluthathione peroxidase, and ceruloplasmin levels imply that the cholesterol/copper challenge engenders pernicious oxidative stress. The origin of this stress is not yet clear, but H_2O_2 generated by Aβ:Cu^{2+} complexes (Aβ1–42>Aβ1–40) (14, 15) is a possibility. Cholesterol serves as a reducing agent by Aβ:Cu^{2+} complexes (15) and modulates the manner in which Aβ:Cu^{2+} complexes insert as oligomers into lipid membranes (10, 11). Thus, excess Cu^{2+} and excess cholesterol may potentiate reactive oxygen species generation caused by Aβ. Inhibition of this reaction is one potential therapeutic action of the metal complexing agent clioquinol, which potently inhibits AD-like amyloid neuropathology in transgenic mice (20) and is currently in clinical trials. Clioquinol crosses the BBB and reacts with copper and zinc in the brain, rather than gut or blood. In contrast, oral treatment of Tg2576 mice with triethylenetetramine (a strong Cu^{2+} chelator that does not cross the BBB) failed to inhibit brain Aβ deposition (20), suggesting that once β-amyloid pathology is established, lowering copper levels in the diet alone is unlikely to be beneficial. However, the findings of Sparks and Schreurs suggest that simultaneous lowering of both dietary copper and cholesterol may have synergistic benefits that are worth exploring further in such preclinical models of AD.

The findings of Sparks and Schreurs will no doubt raise concerns about copper contamination of drinking water as a risk factor for AD and are reminiscent of the concerns of a decade ago about aluminum in drinking water being such a risk factor. Aluminum, like copper, is a prooxidant metal ion. Unlike aluminum, copper is normally abundant in the brain, is enriched in plaques (21), where it has been shown (along with zinc but no other metal ion) to coordinate Aβ (15, 22, 23), and precipitates Aβ at submicromolar concentrations (5, 8). Hence, the biochemical argument for copper exposure being potentially relevant to AD pathophysiology is stronger than the argument for aluminum. The epidemiological studies associating aluminum in drinking water with AD failed to draw a clear consensus to support or exclude an association (24), but the studies that found positive correlations (25) typically did not study other metal ions (except for rare instances of data on calcium and iron, with no clear association). However, the processes that contaminate drinking water with aluminum (whether manmade or geological) could simultaneously cause contamination with other metal salts. Therefore, the findings of Sparks and Schreurs prompt the revisiting of these epidemiological studies while broadening the water analysis to include copper. More immediately, however, it would be important to determine whether copper at low micromolar concentrations in drinking water, under various levels of dietary cholesterol intake, increases AD-like Aβ amyloid plaque neuropathology in transgenic mouse models of AD, such as the Tg2576 model (26).

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1. Bush, A. I. (2003) *Trends Neurosci.* **26,** 207–214.
2. Halliwell, B. & Gutteridge, J. M. C. (1984) *Biochem. J.* **219,** 1–14.
3. Masters, C. L., Multhaup, G., Simms, G., Pottgiesser, J., Martins, R. N. & Beyreuther, K. (1985) *EMBO J.* **4,** 2757–2763.
4. Bush, A. I., Pettingell, W. H., Multhaup, G., Paradis, M. D., Vonsattel, J. P., Gusella, J. F., Beyreuther, K., Masters, C. L. & Tanzi, R. E. (1994) *Science* **265,** 1464–1467.
5. Atwood, C. S., Moir, R. D., Huang, X., Bacarra, N. M. E., Scarpa, R. C., Romano, D. M., Hartshorn, M. A., Tanzi, R. E. & Bush, A. I. (1998) *J. Biol. Chem.* **273,** 12817–12826.
6. Maynard, C., Cappai, R., Volitakis, I., Cherny, R., White, A., Beyreuther, K., Masters, C., Bush, A. & Li, Q.-X. (2002) *J. Biol. Chem.* **277,** 44670–44676.
7. Sparks, D. L. & Schreurs, B. G. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 11065–11069.
8. Atwood, C. S., Scarpa, R. C., Huang, X., Moir, R. D., Jones, W. D., Fairlie, D. P., Tanzi, R. E. & Bush, A. I. (2000) *J. Neurochem.* **75,** 1219–1233.
9. Hesse, L., Beher, D., Masters, C. L. & Multhaup, G. (1994) *FEBS Lett.* **349,** 109–116.
10. Curtain, C., Ali, F., Volitakis, I., Cherny, R., Norton, R., Beyreuther, K., Barrow, C., Masters, C., Bush, A. & Barnham, K. (2001) *J. Biol. Chem.* **276,** 20466–20473.
11. Curtain, C. C., Ali, F. E., Smith, D. G., Bush, A. I., Masters, C. L. & Barnham, K. J. (2003) *J. Biol. Chem.* **278,** 2977–2982.
12. Kontush, A., Berndt, C., Weber, W., Akopyan, V., Arlt, S., Schippling, S. & Beisiegel, U. (2001) *Free Radical Biol. Med.* **30,** 119–128.
13. Zou, K., Gong, J. S., Yanagisawa, K. & Michikawa, M. (2002) *J. Neurosci.* **22,** 4833–4841.
14. Huang, X., Cuajungco, M. P., Atwood, C. S., Hartshorn, M. A., Tyndall, J., Hanson, G. R., Stokes, K. C., Leopold, M., Multhaup, G., Goldstein, L. E., *et al*. (1999) *J. Biol. Chem.* **274,** 37111–37116.
15. Opazo, C., Huang, X., Cherny, R., Moir, R., Roher, A., White, A., Cappai, R., Masters, C., Tanzi, R., Inestrosa, N. & Bush, A. (2002) *J. Biol. Chem.* **277,** 40302–40308.
16. Atwood, C. S., Huang, X., Kahtri, A., Scarpa, R. C., Moir, R. D., Kim, Y.-S., Tanzi, R. E., Roher, A. E. & Bush, A. I. (2000) *Cell. Mol. Biol.* **46,** 777–783.
17. Wolozin, B., Kellman, W., Ruosseau, P., Celesia, G. G. & Siegel, G. (2000) *Arch. Neurol.* **57,** 1439–1443.
18. Fassbender, K., Simons, M., Bergmann, C., Stroick, M., Lutjohann, D., Keller, P., Runz, H., Kuhl, S., Bertsch, T., von Bergmann, K., *et al*. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 5856–5861.
19. Simons, M., Schwarzler, F., Lutjohann, D., von Bergmann, K., Beyreuther, K., Dichgans, J., Wormstall, H., Hartmann, T. & Schulz, J. B. (2002) *Ann. Neurol.* **52,** 346–350.
20. Cherny, R. A., Atwood, C. S., Xilinas, M. E., Gray, D. N., Jones, W. D., McLean, C. A., Barnham, K. J., Volitakis, I., Fraser, F. W., Kim, Y.-S., *et al*. (2001) *Neuron* **30,** 665–676.
21. Lovell, M. A., Robertson, J. D., Teesdale, W. J., Campbell, J. L. & Markesbery, W. R. (1998) *J. Neurol. Sci.* **158,** 47–52.
22. Cherny, R. A., Legg, J. T., McLean, C. A., Fairlie, D., Huang, X., Atwood, C. S., Beyreuther, K., Tanzi, R. E., Masters, C. L. & Bush, A. I. (1999) *J. Biol. Chem.* **274,** 23223–23228.
23. Dong, J., Atwood, C. S., Anderson, V. E., Siedlak, S. L., Smith, M. A., Perry, G. & Carey, P. R. (2003) *Biochemistry* **42,** 2768–2773.
24. Flaten, T. P. (2001) *Brain Res. Bull.* **55,** 187–196.
25. Rondeau, V., Commenges, D., Jacqmin-Gadda, H. & Dartigues, J. F. (2000) *Am. J. Epidemiol.* **152,** 59–66.
26. Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F. & Cole, G. (1996) *Science* **274,** 99–102.